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For: A METHOD AND INSTRUMENTATION FOR MICRO DISPENSATION OF DROPLETS

L E T T E R

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

May 15, 2003

Sir:

Under the provisions of 35 U.S.C. § 119 and 37 C.F.R. § 1.55(a), the applicant(s) hereby claim(s) the right of priority based on the following application(s):

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SWEDEN	0103522-9	October 21, 2001
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A certified copy of the above-noted application(s) is(are) attached hereto.

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Respectfully submitted,

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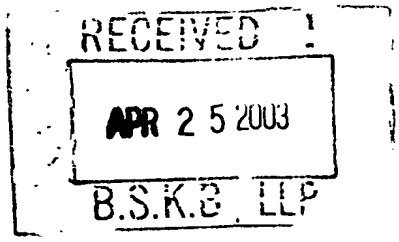

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A METHOD AND INSTRUMENTATION FOR MICRO DISPENSATION OF DROPLETS.

TECHNICAL FIELD

The present invention concerns an interface between the macro and the micro world with respect to the dispensation of droplets of a liquid to a spinning microfluidic device.

In the context of the invention microlitre (µl) range includes the nanolitre (nl) range which includes the picolitre (pl) range.

BACKGROUND TECHNOLOGY AND PUBLICATIONS.

An increasing number of microfabricated analysis and preparative systems have been presented during the last decade. However, the main focus of microscaling has been the analytical and/or preparative performance and in practice very little attention has been paid on interfacing these microworlds with the surrounding macroworld with respect to dispensation of the various liquids that are to be processed within microfluidic devices.

Previous microfabricated analysis and preparative systems have typically been in the form of microfluidic chips, typically containing one, two or more microchannel structures in which a liquid is transported and processed. Variants that can be spun around an axis of symmetry for driving liquid flow have been suggested, e.g. circular forms or discs.

Modifications of traditional ink-jet technology have been suggested to accomplish liquid dispensation to microfluidic devices. In most cases the dispensing unit has been linked to a liquid reservoir without any possibility to use a flow-through system (Sziele at al., "*Adaption of a microdrop injector to sampling in capillary electrophoresis*", J. Chromatogr. A 669 (1994) 254-257; Schober et al., "*Accurate high-speed liquid handling of very small biological samples*", Biotechniques 15 (1993) 2; Nilsson et al., "*Thin-layer immunoaffinity chromatography with bar code quantitation of C-reactive protein*", Anal. Chem. 67 (1995) 3051-3056; Wallace et al.,

"*Ink-jet based fluid microdispensing in biochemical applications*", Lab. Automation News 1(5) (1996) 6-9; and Lemmo et al., "*Characterization of an inkjet chemical microdispenser for combinatorial library synthesis*" Anal. Chem. 69 (1997) 543-551.

Some years ago a versatile through-flow channel microdispenser that could be adapted for dispensation to microfluidic devices was presented (Laurell et al., "*Flow-through sampling cell and use thereof*" **US 6,192,768**, Gyros AB) and later further developed (Laurell et al., "*Design and development of a silicon microfabricated flow-through dispenser for on-line picolitre sample handling*", J. Micromech. Microeng. 9 (1999) 369-376; Thornell et al., "*Desk top microfabrication – Initial experiments with a piezoceramic*", 9 (199) 434-437; Tormod et al., "*Device for dispensing droplets*", **WO 0130500**, Gyros AB) and Stjernström et al., "*A multi-nozzle piezoelectric microdispenser for improving the dynamic volumetric range of droplets*" in Proceedings of μ-TAS 2000 Symposium 14-18 May, 2000, Enschede, the Netherlands, Eds. van den Berg et al., Kluwer Academic Publisher).

The flow-through sampling cell developed by Laurell et al (supra) has been suggested for dispensing droplets to microfludic discs (Ekstrand et al., "*Microfluidics in a rotating CD*" in Proceedings of μ-TAS 2000 Symposium 14-18 May, 2000, Enschede, the Netherlands, Eds van den Berg et al., Kluwer Academic Publisher).

With respect to microfluidic devices that can be spun, dispensation has in practice primarily taken place before any spinning or rotation of the disk or during time periods when the spinning is halted. See for instance the experimental parts of the above-referenced publications. Typically, liquid aliquots in the microlitre (μl) range have been dispensed to individual inlet ports of the various microchannel structures of a disc whereafter spinning has been initiated to assist penetration of the liquid into the structures. This way of dispensation is tedious with respect to other procedures in optimised microfluidic systems and means a considerable risk for disturbing evaporation during the dispensing procedure. This risk increases when going down to dispensation of nl- and pl-volumes. It would be beneficial to design a mico/micro-interface that could accomplish dispensation while spinning the microfluidic chip (provided it can be spun).

Dispensation of liquid aliquots during spinning is associated with targeting problems that are not at hand when the disc is resting because during spinning the target area is moving. When going down in scale, the problem becomes more severe because then also the target area is scaled down and a higher accuracy in targeting will be required. If maintaining the same size of the target area, the risk for undesired evaporation would be increased.

OBJECTS OF THE INVENTION

The main object is to provide an instrument set up (arrangement) and a method that enable dispensation of liquid droplets to inlet ports of individual microchannel structures that are present in a common microfluidic disc while spinning the disc. The spinn speeds are > 0 rpm, for instance ≥ 25 rpm, such as ≥ 50 rpm or ≥ 100 rpm or ≥ 1 000 rpm or even high, for instance up to 15 000 rpm or 20 000 rpm, and/or with uniform droplet sizes in the range of 10^{-6} – 10^{0} µl, such as 10^{-5} – 10^{-1} µl and/or ≤ 10^{-1} µl or ≤ 10^{-2} µl or 10^{-3} µl or 10^{-4} µl.

A subobject is to provide an instrument set up (arrangement) and a method, which enable the transfer of a gradient of a liquid formed in the microworld into the individual microchannel structures of a microfluidic device and to apply the gradient within the device to an experiment that is performed within at least one of the microchannel structures. The term "gradient of a liquid" means that there is a change in composition of the liquid as a function of time. The experiments may be of the same kind as discussed under the heading "Microfluidic Discs and Processes to be Performed" below.

FIGURES

Figure 1a and b illustrate a variant of the instrumentation set up (arrangement) of the present invention.

Figure 2 gives the microfluidic structures of the disc used in experiment 5.

Figure 3 refers to results obtained for experiment 1.

Figure 4 refers to results obtained for experiment 2.

Figure 5 refers to results obtained for experiment 3.

THE INVENTION

Th inventors have carefully valuated the parameters that may influence the trajectory path of a sequence of droplets that are ejected through an orifice towards a spinning surface containing separate target areas for the individual droplets.

The system configuration (**100**) is given in **figure 1a**.

The inlet ports (**101**) (IP^0 I, IP^1 I, IP^2 I etc) of x individual microchannel structures (**102**) are separated on the microfluidic disc (**103**) by an angle α radians. The inlets are at a radial distance r from the centre of the disc, rotating at n rpm (**figure 1b**). The angular velocity ω of the disc is determined by the equation:

$$\omega = 2\pi n/60 \quad \text{in rad/s} \quad \textit{(eq 1)}$$

When a triggering mark (**104**) on the disc passes a fixed trigger position (**105**) in the surroundings, a dispensing signal (**106**) comprising a predetermined number of dispensing pulses with a frequency of f Hertz will be sent to a dispensing actuator (**107**). The number of pulses is equal to the number of structures x into which a droplet is to be dispensed for the next revolution. The actuator (**107**) is associated with an orifice (**108**) in the flow-through channel (**109**) of a dispenser (**110**) so that droplets (**111**) can be ejected towards the surface (**112**) of the disc (**103**) at the frequency of f Hertz. The fixed trigger position (**105**) and the first structure into which the first droplet must enter are separated by an angle of β radians. The orifice (**108**) of the dispenser (**110**) is positioned above the surface (**112**) at a distance of γ radians from the disc trigger position (**105**). The radial position of the orifice is typically the same as for the inlet ports (IP^0 I, IP^1 I, IP^2 I etc) (**101**). Thus it is possible to determine the time T_{trig} between the time at which the triggering mark passes the fixed trigger position and the time at which a predetermined inlet port passes in front of the orifice (after a predetermined number of complete rotations p) by the following equation:

$$T_{trig} = [(\beta+\gamma)/\omega] + [2\pi \cdot p/\omega] \quad \text{in s} \quad \textit{(eq 2)}$$

The dispenser is located at a fixed point, h meters above the disc (typically less than a half centimetre). The droplets are shot at a speed v, which is dependent on the

pulse shape including amplitude and frequency f and the liquid characteristics. T_{elec} is the time period between the time at which the triggering mark passes the triggering position and the time at which the first droplet is ejected through the orifice for the revolution, which is immediately subsequent to the triggering mark having passed the triggering position. The minimum droplet velocity v_{min} can be determined by the equation:

$$v_{min} = h/[((\beta+\gamma)/\omega) + (2\pi \cdot p/\omega) - T_{elec}] \qquad \text{in m/s (eq 3)}$$

If we consider that v is constant throughout the droplet trajectory, the dispensing frequency f required for each droplet to reach the successive inlet port (IP^0 I, IP^1 I, IP^2 I etc) is determined by:

$$f = \alpha/\alpha \qquad \text{in hertz (eq 4)}$$

The first aspect of the invention is a method for dispensing droplets of a liquid to a microfluidic disc comprising a microchannel structure I with an inlet port I (IP^0 I). The method is characterized in comprising steps (i) – (iii) where

Step (i) is to provide (1) a microfluidic disc as defined above which in addition to the microfluidic structures with inlet ports contains a triggering mark from which the angular position of any other part of the disc is defined, and (2) the innovative dispenser arrangement described herein.

Step (ii) is to place the disc in the arrangement and program a controller of the arrangement with values for dispensation parameters which will secure dispensation of a droplet into inlet port I (IP^0 I). The steps of placing and programming are interchangeable.

Step (iii) is to permit the arrangement to proceed with the dispensation.

The invention is described herein in relation to

(a) an arrangement in which the disc is rotating and the drop dispenser is at a fixed position during operation, and

(b) dispensation is taking place in the direction of earth's gravity field (downwards) with the disc placed perpendicular thereto.

There is a number of variants that can be equivalently used in the invention and therefore also are included by the Doctrine of Equivalence. Examples of such variants are:

(a) dispensing the droplets while rotating the dispenser orifice around the axis of symmetry of a microfluidic disc,

(b) dispensing in other directions than the direction of earth's gravity field preferably while maintaining the disc surface perpendicular to the dispensation direction, etc. Dispensation in other direction includes upward dispensation, i.e. against earth's gravity field, and perpendicular to earth' gravity field.

The liquid to be dispensed may be a homogeneous solution or a suspension, emulsion or dispersion. Dispersed/suspended particles may be biological, for instance cells and viruses or parts thereof that are in particle form, solid phases that are in particulate form as described under the heading "Microfluidic Discs and Processes to be Performed". This kind of solid phase typically is dispensed in order to create a packed bed within a microchannel structure.

The microfluidic disc referred to in **step (i)** is further described subsequent to **step (ii)** and **(iii)**. The triggering mark **(104)** is a distinct mark on a rotating part of the disc, preferably associated with the circumference of the disc, for instance the edge or an annular zone close to the edge.

The dispenser arrangement (instrumentation set up) provided in **step (i)** comprises:

a) a spinner **(113)** for rotating the disc **(103)** around its axis,

b) a drop dispenser **(110)** permitting dispensation of droplets **(111)** to inlet port I **(101)** through a dispenser orifice **(108)**,

c) a fixed trigger position **(105)**, and

d) a controller **(114)** which is capable of initiating a dispensation of a droplet into inlet port I **(111)** as a function of the triggering mark **(104)** passing the trigger position **(105)**.

Further details about the various parts of the arrangement are given after the description of step (ii).

The arrangement constitutes a second main aspect of the invention.

For step (ii) the main parameters to be programmed are defined by the equations given above and/or depend on physico-chemical properties of the liquid as such. These parameters include:

(a) speed of rotation of the disc,

(b) the revolutions under which dispensation is to take place,

(c) shape, for instance amplitude, of the dispensing signal,

(d) delay between the signal from the trigger position and the actual dispensing of a droplet,

(e) distance between the dispenser orifice and the disc, and

(f) the frequency of droplet dispensation into inlet port I,

The values of the parameters (a)-(e) are selected to give dispensation of the droplets to inlet port I.

If the manufacturer prefixes a parameter in the arrangement, for instance, the term "programmed" above includes this kind of pre-programming meaning. The most suitable parameters for this kind of programming seems to be (d) and/or (e).

In case the composition of the liquid changes during the dispensation, physico-chemical characteristics of the liquid may also change. This may influence the velocity with which a droplet leaves the dispenser orifice and change the target are for the droplets. See experiment 2 of the experimental part. From example 3 it can be deduced that changing the amplitude of the pulse/pulses of the dispensing signal can compensate a change in velocity during dispensation. Liquid characteristics that may change are surface tension, density etc because of a change in composition (gradient). Equation 2 given above illustrates other variables that can be changed for compensating a change in velocity:

(a) the angular velocity (ω),

(b) the distance (h) between the orifice and the disc surface, and

(c) T_{elec} = delay of the dispensing signal after the triggering mark has passed the triggering position.

Determination of th influence various parameters may have on the droplet velocity can also be elucidated from separate experiments, for instance as illustrated in experiment 2 or from the pattern of where the droplets hit the disc surface during droplet dispensation, e.g. for a liquid containing a gradient. Appropriate changes in the shape of the dispensing pulses can be determined empirically as outlined in experiment 3 of the Experimental Part. Compensation functions or discrete values so found or compensation functions or values derived from equation 2 can then be programmed into the controller either in **step (ii)** or during **step (iii)**. Also manual adaptation of the velocity or trajectory path of the droplets during **step (iii)** can be envisaged.

In the case the microfluidic disc comprises several inlet ports to which droplets are to be dispensed, the dispensing signal comprises several pulses at a frequency, which is determined by the angular distance between the inlet ports and by the angular velocity (ω) of the disc. The number of pulses is equal to the number droplets formed for a dispensing signal and also equal to the number of inlet ports to which a droplet shall be dispensed. Accordingly also in this case the controller is set to values for the parameters (a) - (f) that will match each other so that the individual droplets ejected through the orifice by a dispensing signal will hit their intended inlet port, respectively.

Step (ii) also comprises programming characteristics of the microfluidic disc to be used, for instance number of inlet ports and their angular position and possibly also radial position if the dispenser is radially movable over the disc when placed in the arrangement. The manufacturer preferably does the programming of disc specific characteristics so that the user only needs to program the kind of disc he intends to use.

The spinner

The spinner (**113**) comprises a motor (**115**) and holder (**116**) with shaft (**117**) for rotating the disc around its axis. An encoder (**118**) may be linked to the shaft and grades a revolution into minor parts, for instance into ≥ 10 000 grades such as ≥ 20 000 grades or ≥ 30 000 grades. The encoder may alternatively be associated with the disc.

The spinner should in the preferred variants has a spinning that is regulate with the intervals given under the heading "Objects of the invention".

Drop dispenser and liquid transport to and through the dispenser.

The drop dispenser (**110**) shall be capable of dispensing droplets to inlet port I (**101**) at controllable frequencies and of controllable volumes and velocities through a dispenser orifice (**108**).

The drop dispenser comprises channel (**109**) for transporting liquid to an orifice (**108**) from which droplets can be dispensed. In the typical case the dispensing principles for drop dispensers used in ink-jet printers can be applied to drop dispensers for use in the present invention, if appropriately modified. Compare the discussion under the heading "Background Technology and Publications"

One kind of suitable drop dispenser has a head with a flow-through channel along which there is a dispensing orifice with which a dispensing actuator is associated, for instance with the channel wall essentially opposite to the orifice. See for instance the dispenser given in **figure 1a**. The actuator typically is sensitive to pressure pulses and/or electrical pulses meaning that each pulse of sufficient amplitude will eject a droplet through the orifice. In an advantageous variant, the actuator comprises a piezoelectric element enabling well-defined and short dispensing pulses for the dispensation of droplets. This kind of drop dispensers have been previously been presented. See the publications cited above in the name of Laurell et al., Thornell et al, Tormod, Stjernström et al., and Ekstrand et al. This kind of drop dispenser has been used in the experimental part.

An alternative dispenser variant comprises a liquid transport channel ending in a dispenser orifice and has a dispensing actuator associated with the orifice at an upstream position. The actuator may be ring-formed embracing the liquid flow passing through the channel. In case electrical pulses are used for droplet formation

the ring may comprise a piezoelectric material. This kind of drop dispensers is availabl from Cartesian (England) and Microdrop, and can be used in the present invention if properly modified. Other candidate dispensers are based on the bubble-jet principle developed by Olivetti (Italy).

Flow-through dispensers have the advantage that the composition of the liquid easily can be changed. This can be accomplished by allowing a discrete train of different liquids (stepwise gradient) or a continuous gradient pass through the channel and programming the controller appropriately. When the sufficient amount of droplets of a certain composition has been dispensed, dispensing is halted until next liquid with the desired composition comes into dispensing position. By using flow-through dispensers the replacement of liquid will thus be facilitated. The alternative dispenser variant requires a more complex design and/or complicated procedures for replacing the dispensing liquid.

The drop dispenser may be linked to a pump (**119**) for driving the liquid transport through the channel from one or more reservoirs (**120**) containing the same or different liquids. By including valves (**121**) at the junction of conduits coming from the reservoirs, stepwise gradients can be created and transferred into the microfluidic structures. By associating a gradient pump (**119**) at the junction continuous gradients can be formed.

Typically gradients are defined as a change in salt concentration, kind of salt, pH, composition of solvents and/or some other component/components that interferes/interfere with a biologically or chemical experiment which is carried out within the microfluidic device.

Depending on the receiving structure (IP I) in the microfluidic disc and the kind of process that is to be carried out within the microfluidic structure, the droplets should have a volume within the interval of $10^{-6} - 10^{0}$ µl, for instance within $10^{-5} - 10^{-1}$ µl. The frequency of droplets is typically such that a microchannel structure receives one droplet per revolution or every second or every third revolution or more rarely.

The optimal velocity of the droplet when leaving the dispenser orifice depends on many factors but should as rule of thumb be found in the interval 0.5-25 m/sec, such as 1-10 m/sec.

Trigger position

The trigger position (**105**) is a fixed position placed outside the disc on a non-rotatable part. The position of the trigger position is typically such that it comes relatively close to the edge of a microfluidic disc that is spun. The trigger position comprises a detector that is capable of detecting the trigger mark (**104**) on the disc (**103**) each time the mark passes the trigger position. Passage of the triggering mark initiates a triggering signal to the controller.

Controller

The controller (**114**), for instance comprising electronic and programmable control means with operator's interface and software, not further disclosed. The controller may be a separate physical part within the arrangement and/or may have parts that are physically associated with the units with which it communicates by sending and receiving signals. The controller (**114**) communicates with the spinner (**113**), the drop dispenser (**110**) and the trigger position (**105**).

The controller is capable of sending the dispensing signal (**106**) to the dispenser (**110**) after having received a triggering signal from the trigger position (**105**). The characteristics of the dispensing signal are defined by values programmed in **step (ii)** including values preset, for instance by the manufacturer, or programmed during **step (iii)**. The controller also controls when the dispensing signal (**106**) shall actuate the dispenser, i.e. when the factual dispensation is taking place. A preferred way is to link the dispensing signal to the angular distance between the triggering mark (**104**) and the trigger position (**104**). If an encoder is linked to the spinning movement as discussed above, the encoder signal can be used to determine when an inlet port I is in position for dispensation and also regulate so that the factual dispensation takes place at the most appropriate time. If the encoder is high-resolving as suggested above, dispensation can take place with a high accuracy with respect to timing.

Alternatively the pre-programmed angular velocity of the disc can be used to calculate the time at which the dispensing signal shall actuat the dispenser.

Microfluidic Discs and Processes to be Performed

The term "a microfluidic disc" means a disc, which comprises a microchannel structure through which one or more liquid aliquots (droplets) are transported and processed in various kinds of microcavities (reaction microcavities). The result of the processing is measured in one or more detection microcavities through corresponding detection areas, which are placed in either or both of the surfaces of the disc. Reaction microcavities and detection microcavities may coincide. The interior of a microchannel structure is in contact with ambient atmosphere through inlet and/or outlet openings and/or vents. Other parts of the structures are normally separated from direct contact with ambient atmosphere by the material of the disc.

The disc concept includes circular discs, discs with an n-numbered axis of symmetry (C_n) where n is an integer 3, 4, 5, 6 or larger and discs with one planar surface and an opposite non-planar surface, for instance a cone with a planar base surface.

A microfluidic disc typically comprises one, two or more microcannel structures, such as ≥ 10, or ≥ 50 or ≥ 100 microchannel structures. The structures may be identical or different. For discs in which there is a plurality of the structures, the structures may be identical or different, for instance with at least one of the structures being different from the other. The inlet port (IP I) used for dispensation according to the invention typically is located at the same radial distance for more than one microchannel structures. The microchannel structures in a disc may be arranged in subgroups such that IP I for each subgroup is at the same radial distance but for different subgroups at different radial distances.

The term "microchannel structure" contemplates that the structure comprises one or more cavities/chambers and/or channels that have a cross-sectional dimension that is ≤ 10^3 μm, preferably ≤ 10^2 μm. The volumes of cavities/chambers are typically ≤ 1000 nl, such as ≤ 500 nl or ≤ 100 nl or ≤ 50 nl or ≤ 25 nl. This in particular applies to the detection and/or reaction microcavities. Chambers/cavities directly connected to

Inlet ports for liquids may be considerably larger, .g. microchambers/microcavities intended for application of sample and/or washing liquids. Microformat means that the liquid aliquots that are transported within the device has a volume in the µl-range, i.e. ≤ 1000 µl such as ≤ 100 µl or ≤ 50 µl and includes the nl-range (nanoformat), such as ≤ 500 nl or ≤ 100 nl or ≤ 50 nl or ≤ 10 nl or less.

The transport of liquid within the microchannel structures may be driven by various forces, for instance inertia force such as centrifugal force, electrokinetic forces, capillary forces, hydrostatic forces etc. Pumps of various kinds may be used. Typically centrifugal force and/or capillary force are utilized at inlet ports.

The disc may be made from different materials, such as plastic material, glass, silicone etc. Polysilicone is included in plastic material. From the manufacturing point of view plastic material is many times preferred because the costs for this kind of material are normally low and mass production can easily be done, for instance by replication. Typical examples are embossing, moulding etc followed by attaching a top lid covering the open microchannel structures so obtained. See for instance WO 9116966 (Pharmacia Biotech AB, Öhman & Ekström). This manufacturing process result in open microchannel structures as an intermediate product which subsequently is covered by a lid, for instance according to the procedures presented in WO 0154810 (Gyros AB, Derand et al) or by methods described in publications cited therein. The hydrophilic/hydrophobic balance are preferably obtained according to the principles outlined in WO 0056808 (Gyros AB, Larsson et al) and WO 0147637 (Gyros AB, Derand et al). All three WO publications are hereby incorporated by reference.

In each microchannel structure, liquids are processed in order to carry out various miniaturised chemical and biological experiments, i.e. assay protocol, synthesis protocol, cell culturing protocols etc within the chemical and biological field including biochemistry, chemistry, biophysics, microbiology, medicine, zoology, molecular biology etc. Processing includes that various chemical reactions and/or biochemical reactions and/or biological reactions etc are taking place. Typical protocols utilise specific reactions between reactants having mutual affinity to each other leading to

(a) formation of an affinity complex that is immobilized to a solid phase in a detection and/or reaction microcavity or

(b) one or more other reaction products that may be soluble or insoluble in the detection microcavity.

Typical detection principles are based on radioactivity, fluorescence, chemiluminescence, bioluminescence, enzymatic activity, chromogens, light scattering (turbidometry) etc, for instance by utilizing reactants that exhibits groups providing the corresponding properties or groups that can be transformed to one of these groups.

Typical reactants in this context are individual members of affinity pairs such as (a) antigen/hapten and the corresponding antibody including its antibody active fragments, (b) lectin and the corresponding carbohydrate structure, (c) native ligands and the corresponding native receptors, (d) complementary nucleic acids including synthetic variants such as synthetic oligonucleotides, (e) Ig(Fc)-binding proteins and Protein A, Protein G and other Ig(Fc)-receptors, (f) ion pairs of opposite charges, enzyme and the corresponding substrate, inhibitor, cofactor, coenzyme etc that can bind to the enzyme, (g) ligand and receptors that are involved in cell surface interactions etc Synthetic variants that more or less mimic a member of a native affinity pair are also included.

The reaction microcavity may contain a separation medium in the form of a porous bed through which a sample liquid containing at lest a substance that is capable of binding to the bed under the conditions applied is passing. During the passage, the substance(s) becomes (become) bound and non-binding substances pass through. Subsequently an eluent may be applied through the bed so that one or more of said at least one substance are released from the bed. Further processing may take place on one or more of the non-binding substances after their passage through the bed, and/or on one or more of said at least one substance while being bound to or subsequent to their release from the bed. Possibly one or more washing liquids may be passed through the bed after the sample liquid but before the eluent. The various liquids used in this kind of protocol may be applied through the same inlet port or through different inlet ports. At least one of the liquids is dispensed to an inlet port in

accordance with the invention. The eluent can be in the form of a continuous or a stepwise gradient of the kind discussed elsewhere in this text.

Further processing may be detection of a substance bound to the bed or of non-binding substance passing through the bed.

The porous bed discussed above may be a porous monolith or a packed bed of porous or non-porous particles. The population of particles defining the bed may be in bead form and be monosized (monodispersed) or polysized (polydispersed). By the term monosized is meant that 95 % of the particles are within the interval of the mean particle size ± 5 %. Populations having other particle distributions are polysized.

The microchannel structures (**200**) used in experiments 4-5 of the Experimental Part is given in **figure 2**. The arrow (**201**) indicates the upward direction and is directed towards the centre of the disc on which the structure is placed. The complete structure used in the experiments comprises a common distribution channel (**202**) with an inlet port (**203**) and an inlet microcavity (**204**) with parallel grooves/ridges (**205**) in the bottom, and an outlet port (**206**). Along the distribution channel (**202**) there is a number of Y-shaped structures (microchannel structures) (**200**) with one of the upward shanks (**208**) being connected to the distribution channel (**202**) and the other upward shank (**209**) comprising an inlet port (**210**) of the same kind as inlet port (**203**). The lower shank (**211**) of the Y-shaped structure contains an outlet port (**212**) opening to ambient atmosphere and has a shallow part (**213**) and a deeper part (**214**). The dual depth means that if a liquid containing particles with a larger diameter than the depth of the shallow part is transported through the structure, the particles will assembly as a packed bed in the deeper part (**214**) immediately upstream the shallow part (**213**). The common distribution channel (**202**) comprises vents (**215**) to ambient atmosphere between the individual microchannel structures. The inner surfaces of these vents are hydrophobized in order to prevent leakage of liquid. There are valves (**216**) in form of hydrophobized inner surfaces between each microchannel structure and the common distribution channel.

In the experiments 4 and 5 below the part occupied by the packed bed of the deeper part (214) corresponds to a reaction microcavity and to a detection microcavity.

The part of the structure that is above the common distribution channel was not used in any of experiment 1 and 2 and is therefore not further described.

The invention is further defined in the patent claims that are part of the description. The invention will now be illustrated in the Experimental Part.

EXPERIMENTAL PART

The microfluidic disc used had been manufactured in transparent plastic material by injection moulding and covered by a lid as outlined under the heading "Microfluidic Disc and Processes to be Performed".

EXPERIMENT 1. Investigation of the flow profile deformation occurring between the pump and the dispenser.

Experimental: The experimental set up was as in figure 1 except that the droplets were not collected in the microfluidic device. Colorimetry, with Cibacron dye (Brillant Red 4B-E, Ciba) in water was used to investigate gradients (from water up to 70% of the Cibacron solution applied over 1, 2 or 3 minutes) with different flow rates (0.1 and 0.5 ml/min). The process was monitored by collecting 500 droplets at predetermined intervals and measuring the colour intensity of the collected samples.

Results: Figure 3 shows four gradients obtained from the dispenser. The result shown demonstrates the reproducibility of a 1 minute gradient at a flow rate of 0.3ml/min. This graph shows that the gradient is obtained after 90 seconds indicating a lag time of 30s within the current configuration.

EXPERIMENT 2. *Velocity of the droplet as a function of the gradient with a constant pulse.*

Experimental: The experimental set up was as in figure 1 except that the droplets were not collected in the microfluidic device. The variation in drop velocity *v* (m/s) with an acetonitrile gradient (0-80%) or a salt gradient 250 mM Tris-HCl pH 8 (0-1.5 M NaCl) was studied. The velocity was measured using a computer IR-camera system (Sydat Automation, Sweden) developed for evaluation of ink-jet print heads.

Results: Se figure 4. The physical properties of the dispensed liquid vary with the gradient profile and clearly affect the velocity of the droplets, leading to misalignment. However, this misalignment can be compensated for by adjusting various parameters, for example the trigger delay or the disc angular velocity (see equations 2 & 3) can be modified, although adjusting the angular velocity may affect flow control (see also Figure 5).
For comparison when the disc is spun at 1500 rpm, the velocity of a point over the disc at a distance of 30 mm from the centre is 4.7 m/s.

EXPERIMENT 3. Velocity of the droplet as a function of the pulse amplitude.

Experimental: The experimental set up was as in figure 1 except that the droplets were not collected in the microfluidic device. The drop velocity *v* (m/s) was studied as a function of the pulse amplitude for normal and high salt buffer solutions.

Results: By adjusting the pulse amplitude it is possible to adjust the drop velocity and thus to solve the misalignment problem.

EXPERIMENT 4. Stepwise elution of Cy5 labelled angiotensin I from nanoliter columns on a disc.

Experimental: C lumns (30 and 35 nanoliters in volume) were packed with SOURCE 15RPC (Amersham Pharmacia Biotech, Sweden) by centrifugation into disc microstructures. The microchannel structures used are given in figure 2. Packing was accomplished by filling a suspension of the bead material into inlet ports (**210**). Upon centrifugation, the beads collect in the deeper part of the lower shank (**214**) of the Y-shaped structure. Peptides (angiotensin I and II) were labelled using a Cy3 or Cy5 labelling kit (Amersham Pharmacia Biotech, Sweden). The columns were conditioned by applying 2 x 500 nl 50% acetonitrile, 0.1% TFA and washed twice with 500 nl of 0.1% TFA under controlled spinning (1500 rpm) by filling the common distribution channel (**202**) via inlet port (**203**) and spinning for each solution. A 500 nl mix of Cy5-labelled angiotensin I (110 nM – Sigma) and Cy3-labeled angiotensin II (880 nM – Sigma) was loaded via inlet port (**203**) to the common distribution channel (**202**) and a stepwise increase in spin speed. Bound components were eluted with a step gradient between 12.5 - 37.5% acetonitrile. Portions corresponding to increasing concentrations of acetonitrile were filled into the common distribution (**202**) by pipetting and passed through the columns by spinning the disc. The acetonitrile concentration was increased by 2.5% in each 200 nl step and applied with a controlled spin flow (1500 rpm). The separation in the columns was monitored using a fluorescence microscope.

Results: The separation of labelled peptides and free dyes has been reproduced successfully on 16 columns processed simultaneously on a disc (results not shown). This result is not linked to the dispensation of the solutions by pipetting. One can envisage that dispensation can also take place according to the invention by droplet dispensation to inlet ports (**210**).

EXPERIMENT 5. Gradient elution on a nanoliter column in a disc.

Experimental: The experimental set up was according to figure 1. Columns (34 and 38 nanoliters in volume) were packed and conditioned as described in example 4. A mix of Cy3 (700 nM)/Cy5 (300 nM) dye (500 nl) was loaded by a stepwise increase in spin speed. The continuous gradient elution was made using a dispenser with a

nozzle 60 µm in diameter and a flow-through channel 1 mm wide and 50 µm in depth. Dispensation of the gradient was according to the invention through inlet port (**210**). Various elution profiles were tested using a dispenser frequency of 1kHz and spinning between 2500 and 1800 rpm. The separation was monitored in the columns by a fluorescence microscope.

Results: The results indicated the beginning of a separation of Cy3 and Cy5 dyes using a gradient 0-40% acetonitrile, 0.1% TFA over 1 minute. Other experiments have shown the possibility to dispense a gradient while spinning into 14 microstructures using various liquids such as water, acetonitrile mixes and Tris buffer (1kHz pulse and 1500 rpm).

This example illustrates that a detector that is capable of detecting the reaction in a reaction and/or detection microcavity can be linked to the arrangement and used to monitor the proceedings of the reaction. In this case reaction is adsorption/desorption to the separation media. The detectors used may be of the same kind as outlined in our copending patent application SE 0103118-6 filed September 17, 2001 (Gyros AB, Magnus Ljungström et el) that hereby is incorporated by reference.

CLAIMS

1. A method for dispensing droplets of a liquid to a microfluidic disc comprising a microchannel structure I with an inlet port I (IP^0 I), **characterized** by comprising the steps of:
   i) providing (1) said disc which has a triggering mark, preferably in the circumference, and (2) a dispenser arrangement comprising:
      a) a spinner for rotating the disc around its axis,
      b) a drop dispenser permitting dispensation of droplets to inlet port I,
      c) a fixed trigger position outside the disc, and
      d) a controller which is capable of triggering the dispensation of a droplet into inlet port I as a function of the triggering mark passing the trigger position;
   ii) placing the disc in the spinner and programming the controller with values for the parameters:
      (a) speed of rotation,
      (b) revolutions under which dispensation is taking place
      (c) shape, for instance amplitude, of the dispensing signal,
      (d) delay between the signal from the detector and the actual dispensing of a droplet,
      (e) distance between the dispenser orifice and the disc, etc, and
      (f) frequency of droplet dispensation into inlet port I,
      the values for (a)-(d) being selected to give dispensation of the droplets to inlet port I
   iii) dispensing the droplets according to the programmed values of step (ii).

2. The method of claim 1, **characterized** in that the liquid comprises a gradient with respect to at least one of its constituents, said gradient being a continuous or a stepwise gradient, for instance containing one, two or more steps.

3. The method of claim 2, **characterized** in that the value for at least one of said parameters (a)-(f) is adjusted during the dispensation to compensate for the

change in velocity of the droplets which possibly is caused by th gradient, said adjustment preferably being handled by the controller.

4. The method of any of claims 1-3, **characterized** in that said microchannel structure I comprises a microcavity positioned downstream to inlet port I and used for carrying out an chemical or biological experiment.

5. The method of claim 4, **characterized** in that said liquid comprises a gradient which is defined as a change in salt concentration, kind of salt, pH, composition of solvents and/or some other component/components that interferes/interfere with an experiment which is carried out in the microcavity.

6. The method of any of claims 4-5, **characterized** in that the microcavity contains a separation media in form of a porous bed, for instance a porous monolith or a packed bed of porous or non-porous particles that may be in beaded form and/or are monosized (monodispersed) or polysized (polydispersed).

7. The method of claim 6, **characterized** in that the method comprises
   a) dispensing a liquid sample to a sample inlet port of microchannel structure I, which sample contains at least one substance that is capable of binding to the bed when passing through it, and
   b) subsequently dispensing an eluent to an inlet port for releasing at least a portion of said substance from the separation medium,
   at least one of said inlet ports being inlet port I and at least the liquid introduced via this port being dispensed as defined by parameters (a)-(e).

8. The method of claim 7, **characterized** in that the inlet port for the eluent is inlet port I and that said eluant is dispensed as defined by the programmed values for the parameters (a)-(f).

9. The method of claim 8, **characterized** in that the eluent comprises a gradient with respect to one of its constituents.

10. The method of any of claim 1-9, **charact rized** in that the time at which the dispensing signal is given depends on the angular distance between the triggering mark and the detector.

11. The method of claim 10, **characterized** in that
    a) the spinner is linked to an encoder which gives at least 10,000 grades, such as at least 20,000 or at least 30,000 grades per revolution, and
    b) the angular distance is determined by the number of grades between the triggering mark and the detector given by the encoder.

12. The method of any of claims 1-11, **characterized** in that the time at which the dispensing signal is given is linked to the speed of rotation.

13. The method of any of claims 1-12, **characterized** in that the signal for dispensing is electrical.

14. The method of any of claims 1-13, **characterized** in that a piezo-driven actuator drives the dispenser which is actuated according to the dispensing signal.

15. The method of any of claims 1-14, **characterized** in that the dispenser is a flow-through dispenser.

16. The method of any of claims 1-15, **characterized** in that the disc comprises one, two or more additional microchannel structures, each of which has an inlet port (IP^1 I, IP^2 I, IP^3 I etc) which correspond to inlet port I (IP^0 I) and are at the same radial distance from the disc centre as inlet port I (IP^0 I).

17. The method of claim 16, **characterized** in that the angular distances between inlet ports (IP^0 I, IP^1 I, IP^2 I, IP^3 I etc) that are located next to each other are the same or different.

18. The method of any of claims 16-17, **characterized** in that said one, two or more additional microchannel structures and microchannel structure I are identical or at least one of them is different from the other.

19. The method of any of claim 16-18, **characterized** in that the dispensing signal comprises a number of pulses such that each droplet formed will correspond to a pulse and that the programmed values for parameters (a)-(f) will be such that for each signal only one droplet will be dispensed into an inlet port.

20. An arrangement for dispensing liquids to a microfluidic disc comprising a microchannel structure with an inlet port I in the surface and a triggering position, preferably in or close to the circumference, **characterized** in that it comprises:

a) a spinner for rotating the disc around its axis,

b) a drop dispenser permitting dispensation of droplets to inlet port I,

c) a fixed trigger position outside the disc, and

d) a controller which is capable of triggering the dispensation of a droplet into inlet port I as a function of the triggering mark passing the trigger position;

ABSTRACT

A method for dispensing droplets of a liquid to a microfluidic disc comprising a microchannel structure I with an inlet port I (IP^0 I). The method is characterised by comprising the steps of:

i) providing (1) said disc which has a triggering mark, preferably in the circumference, and (2) a dispenser arrangement comprising:
   a) a spinner for rotating the disc around its axis,
   b) a drop dispenser permitting dispensation of droplets to inlet port I,
   c) a fixed trigger position outside the disc, and
   d) a controller which is capable of triggering the dispensation of a droplet into inlet port I as a function of the triggering mark passing the trigger position;
ii) placing the disc in the spinner and programming the controller with values for the parameters:
   (a) speed of rotation,
   (b) revolutions under which dispensation is taking place
   (c) shape, for instance amplitude, of the dispensing signal,
   (d) delay between the signal from the detector and the actual dispensing of a droplet,
   (e) distance between the dispenser orifice and the disc, etc, and
   (f) frequency of droplet dispensation into inlet port I,
   the values for (a)-(d) being selected to give dispensation of the droplets to inlet port I
iii) dispensing the droplets according to the programmed values of step (ii).

The invention comprises also the arrangement as such.

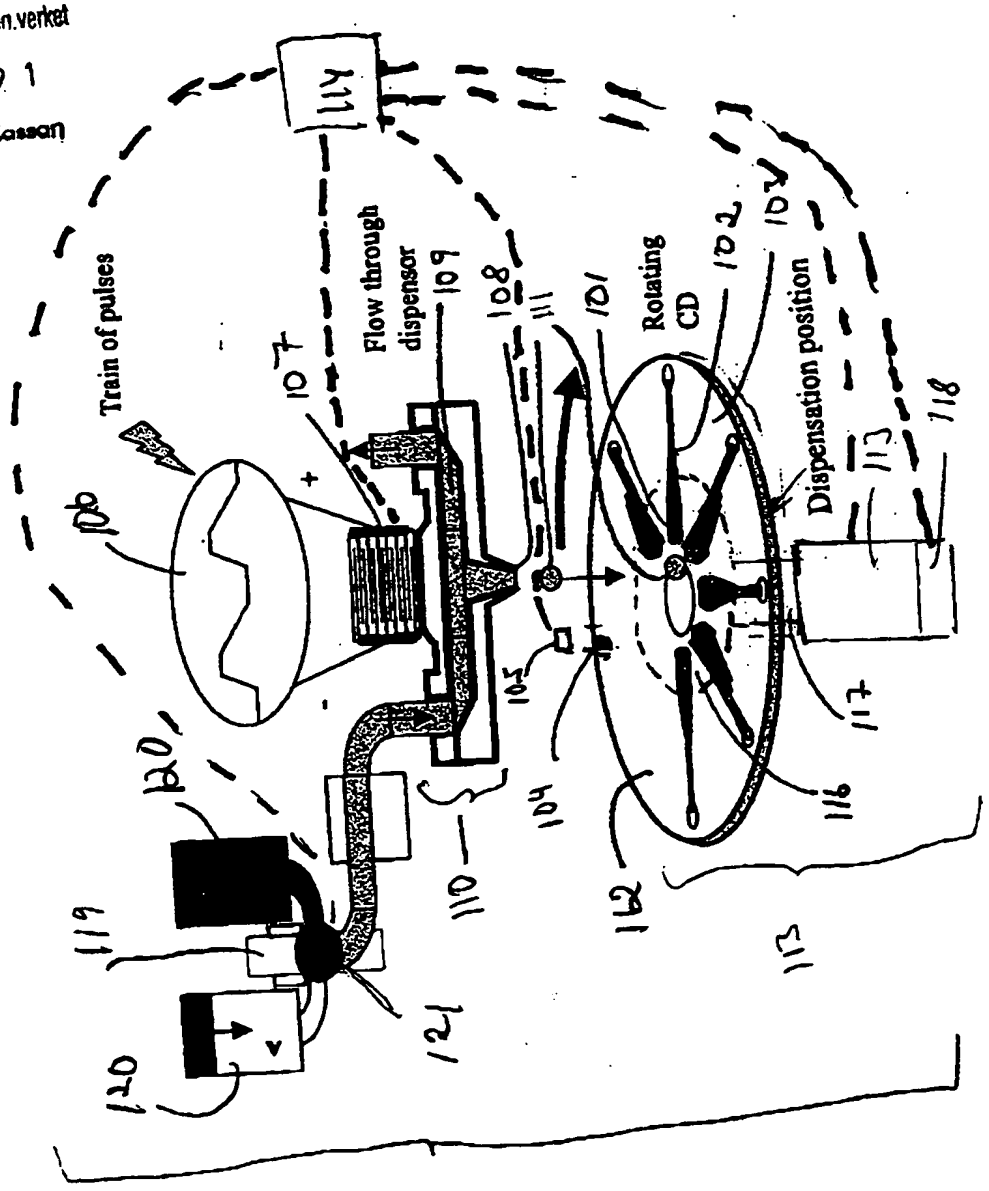
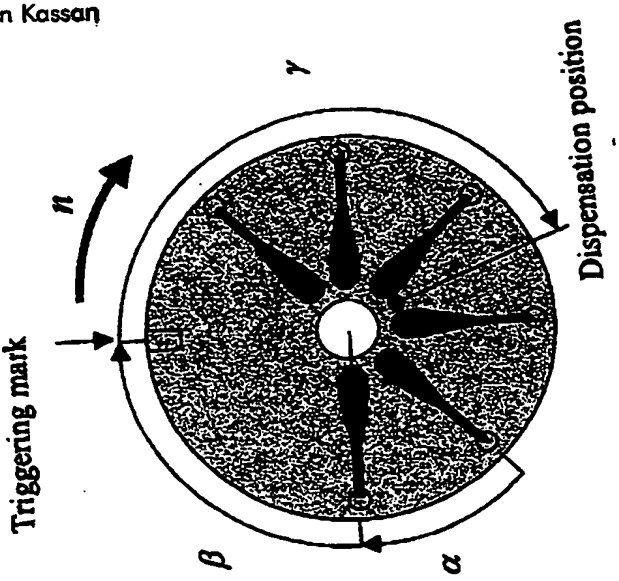


Figure 1a

Figure 1b



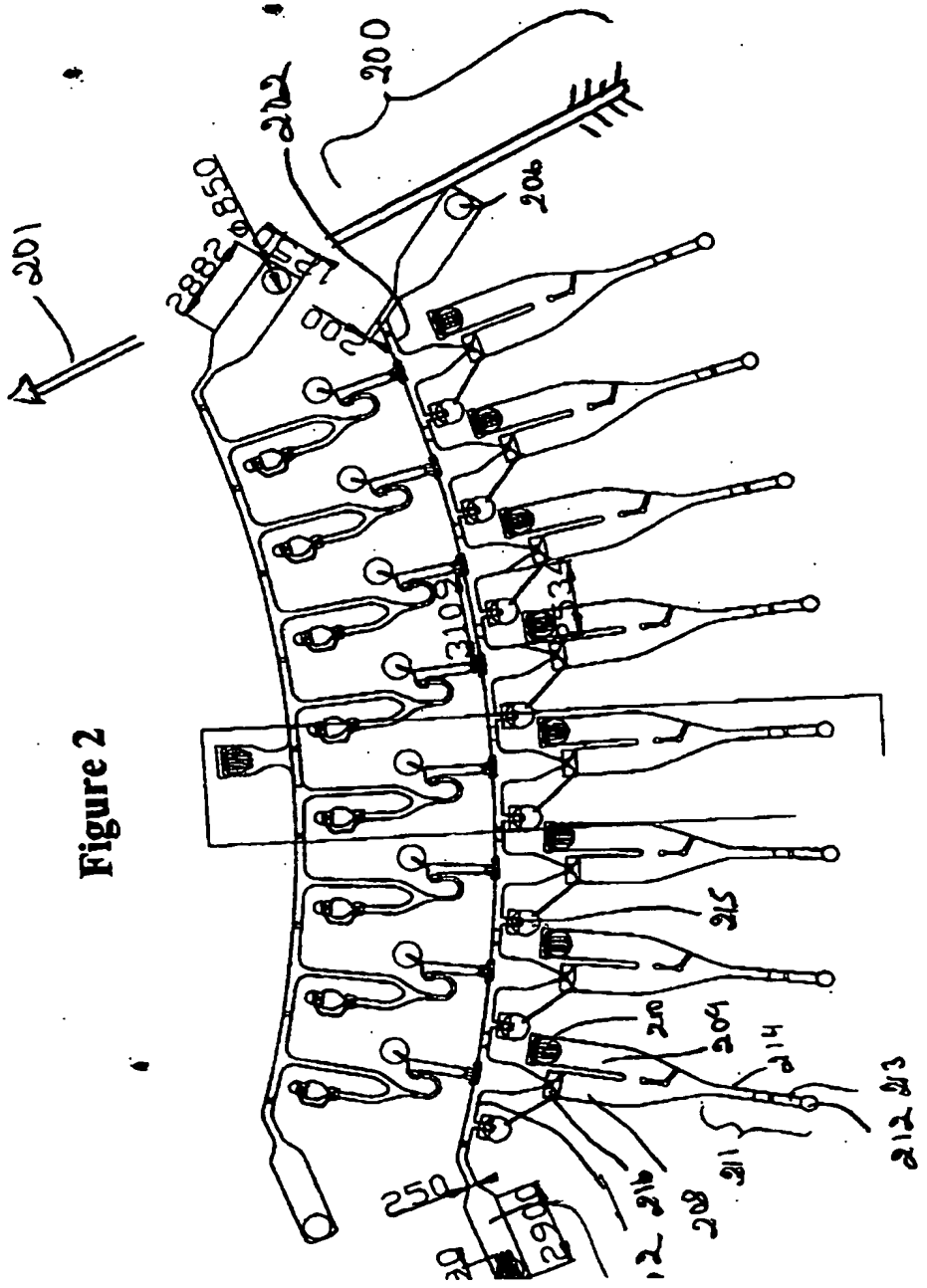


Figure 2

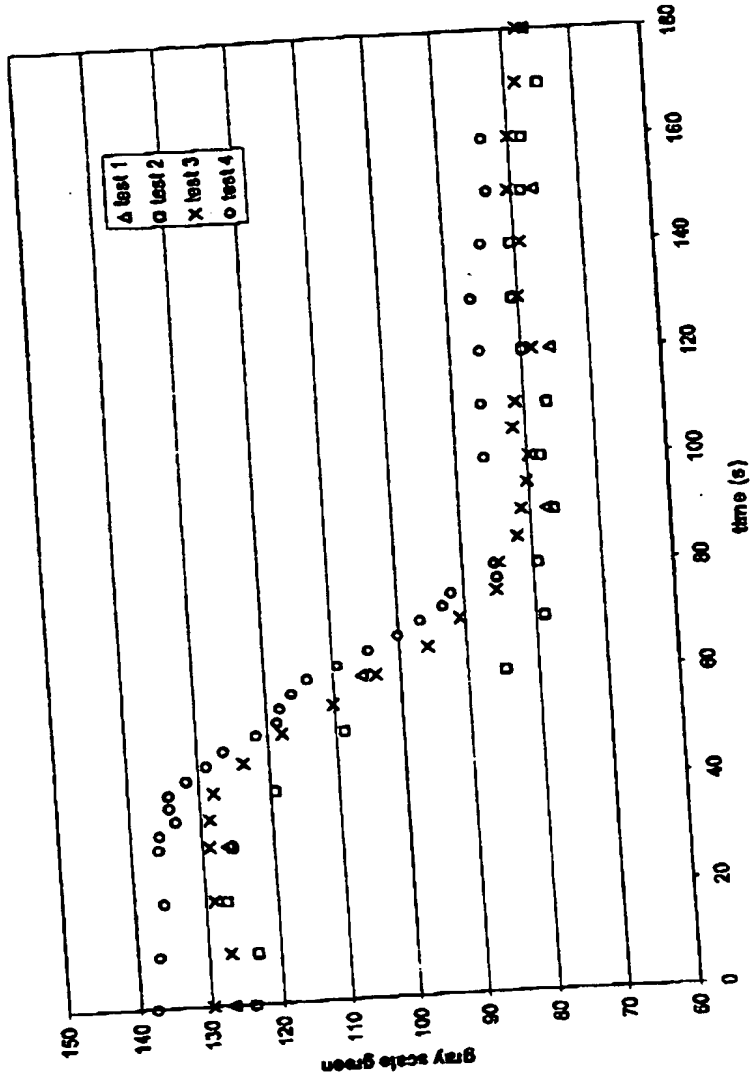


Figure 3

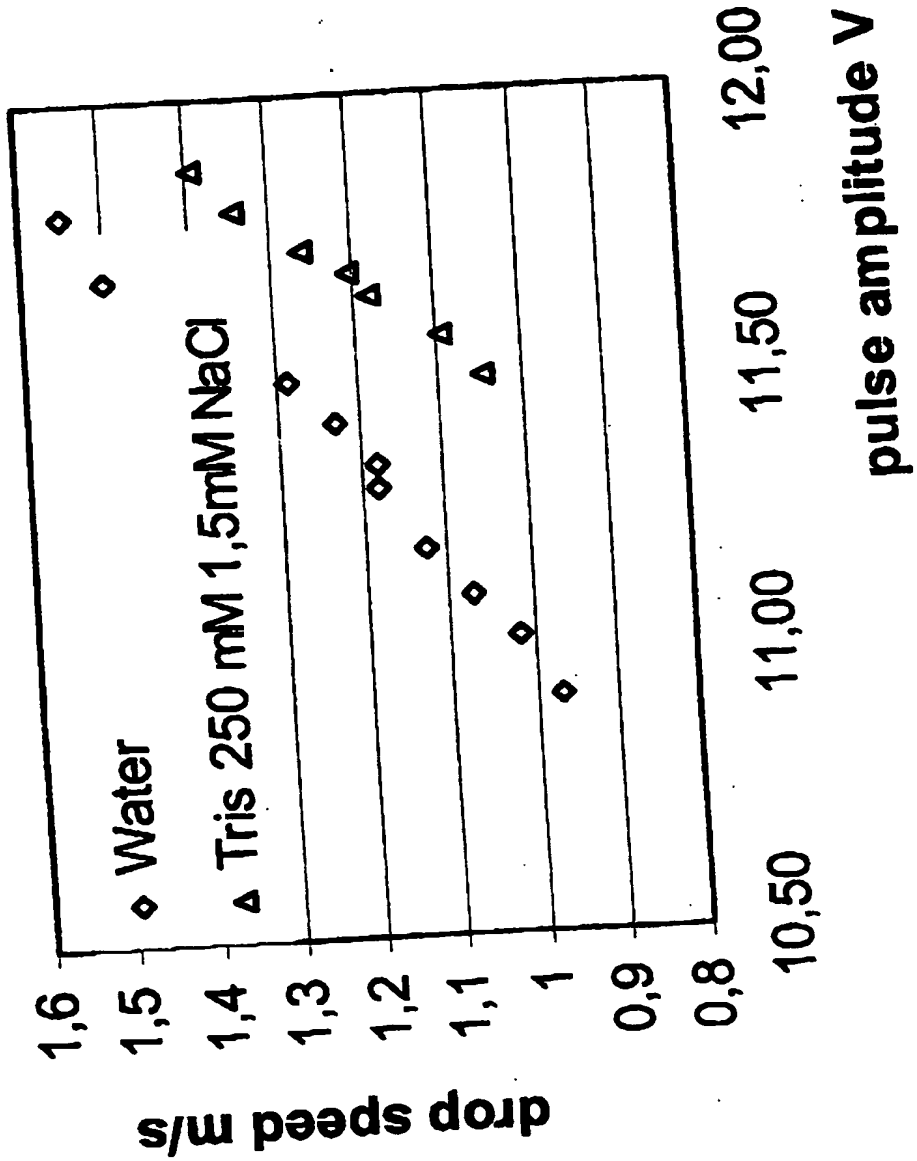


Figure 4

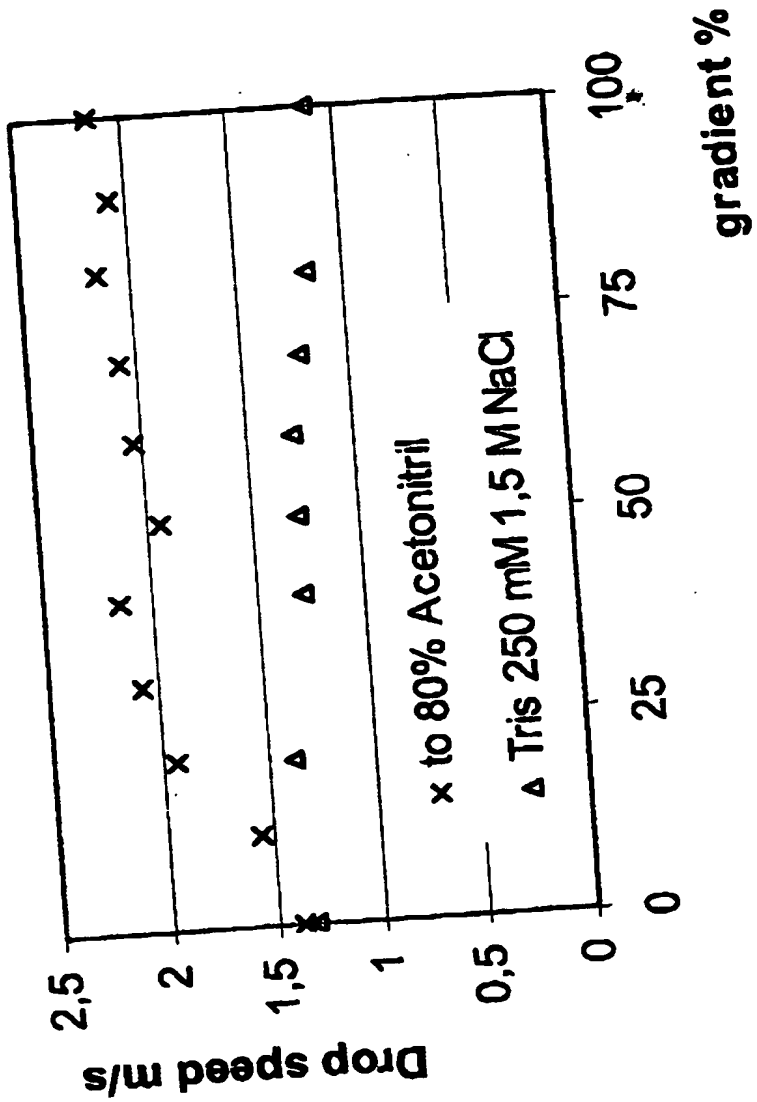


Figure 5